Examiner's rejection, in the interest of expedited prosecution of the application, applicant has amended Claims 1 and 12 to delete the term "substantially" as suggested by the Examiner.

b. Claims 19-25 are rejected as being indefinite in step (c) for reciting "determining the hybridization pattern." Without acquiescing in the rejection, applicant has cancelled claims 19-25 without prejudice to prosecution of these claims in a further continuation or divisional and therefore believes the rejection is moot.

Rejection of Claims 1-25 under 35 U.S.C. §103(a).

The Examiner rejects claims 1-25 under 35 U.S.C. §103(a) as being unpatentable over Cantor et al., (U.S. Patent No. 5,795,714) (hereinafter "Cantor") in view of Southern et al., (Genomics, 1992, 13:1008-1017) (hereinafter "Southern").

As to independent Claim 1, the Examiner alleges that Cantor teaches a method of determining the presence of a mutation in a target polynucleotide (Example 5) comprising the steps of providing a polynucleotide array wherein each probe comprises a double stranded region and a single stranded n-mer overhang region such that the overhangs in each array constitute a complete set of n-mers; hybridizing a target polynucleotide to said overhangs in the array to generate a target hybridization pattern and determining the presence of a mutation in the target polynucleotide by analyzing hybridization patterns. The Examiner further alleges that Cantor teaches the method for detecting mutations and comparative sequencing. The Examiner states that Cantor does not teach providing at least two identical polynucleotide probe arrays wherein the target polynucleotide is hybridized to one array and a reference polynucleotide is hybridized to a second array and wherein the presence of a mutation is determined by comparing the target and reference hybridization patterns. However, the Examiner alleges that Southern teaches a method for analyzing and comparing nucleic acid sequences by hybridization to arrays wherein the analysis and comparison of hybridization patterns determines the presence of a mutation. The Examiner further alleges that Southern teaches the method would be applicable in the analysis and comparisons of gene sequences which are known to be affected by mutations e.g. p53 and CFTR.

Applicant respectfully traverses the Examiner's rejection for at least the following reasons. First, the Examiner alleges that Example 5 of the '714 teaches a method of determining the presence of a mutation in a target polynucleotide. Applicant respectfully submits that this characterization of Cantor is inaccurate. While Example 5, Table 2 and Table 3 all discuss matched and mismatched duplexes, the purpose is not, as suggested by the Examiner, to determine the presence of a mutation in a target polynucleotide. Rather, the stated purpose is to attempt to demonstrate that the invention of Cantor does not suffer (or suffers less) from one of the drawbacks of prior art methods of sequencing by hybridization (SBH). Column 2, lines 57-59 of Cantor states "A second drawback (of SBH) is the poor level of discrimination between a correctly hybridized, perfectly matched duplexes (*sic*), and an end mismatch." In sequencing by hybridization schemes, the data gathered to determine the sequence of the target must be limited to perfectly matched duplexes or the sequence will be inaccurate. If duplexes comprising mismatch bases are scored as a match, the user will be unable to determine the identity of the base or worse, the user will determine that the wrong base occupies the mismatch position, thus creating an error in the sequence. In Example 5, Cantor asserts DNA ligases are used "to covalently attach hybridized target nucleic acid to the *correct* immobilized oligonucleotide probe." (Column 22, lines 40-42, emphasis added.) With respect to Tables 2 and 3, the specification further states, "Table 2 looks at the effect of the position of the mismatch and Table 3 examines the effect of base composition on the relative discrimination of perfect matches verses weakly destabilizing mismatches. These data demonstrate that .... the serious problems of dealing with base composition effects on stability seen in ordinary SBH do not appear to be a problem for positional SBH..." (Column 22 line 67 and column 23 lines 1-11) The same paragraph further states that "any mismatches that survive in this position will be *eliminated* by a polymerase extension reaction..." (Column 23 lines 13-15, emphasis added.)

Cantor does discuss the analysis of mutations in Example 13. However, applicants assert that this discussion is limited to the use of "custom arrays of probes" as this is, in fact the suggested title of Example 13 (see Column 30, line 52). Custom or customized arrays are discussed Column 12, lines 19-56 and specifically defined at Column 12, lines 20-34 which state: "(probes are created by) synthesizing a plurality of

single-stranded first nucleic acids and an array of longer single-stranded second nucleic acids... to form hybrids having a double-stranded portion...hybridizing a single-stranded nucleic acid *target* to the hybrids, ligating the hybridized target to the first nucleic acid of the hybrid, isolating the second nucleic acid (thereby isolating only those second nucleic acid strands which hybridized to the target)...(and hybridizing the first nucleic acid to this isolated second nucleic acid) to form a nucleic acid probe." (emphasis added). Because the pool of probes is chosen by isolating only those probes which hybridized to the nucleic acid target, applicants assert that these arrays consist of only those probes which are complementary to the particular target sequence, and therefor do not, in any way constitute a complete n-mer array.

Applicant respectfully asserts that Cantor does not, in fact, teach or discuss the use of a complete n-mer to analyze mutations.

Furthermore, applicants respectfully submit that Southern does not in fact teach a method for analyzing and comparing sequences by hybridization to arrays wherein the analysis and comparison of hybridization patterns determines the presence of a mutation. Applicant respectfully submits that the Southern reference is a speculative article discussing the types of analyses which might be performed once further, significant research is performed in order to determine whether the proposed scheme is viable. The Abstract states "Further development is needed before the method can be used routinely..." (Page 1008, first column, first paragraph, lines 9-10.) The Discussion states on Page 1014, second column, first full paragraph, lines 5-7, "...several problems must be solved before the method can be put to practical use." Southern goes on to discuss the problems which must be resolved. Applicants respectfully submit that these problems could not be resolved without undue experimentation.

Chief among the problems to be resolved is the fact that the array of Southern comprises only oligopurines, that is, only T's and C's. The effect of this is discussed on the final paragraph of page 1014 which carries over to page 1015. In this paragraph, Southern discusses the potential problems created by foldback sequences, repeats and long homopolymer runs. The text continues to say that such problems "must be eliminated or taken into account in the interpretation of the data." (Page 1015, first column, carry-over paragraph lines 7-8.) The text goes on to suggest that the "rules are

likely to be complex...(and) they will emerge from detailed analysis...." While the text speculates that a complete set of octanucleotides could be used to compare sequences and find single base differences, applicant respectfully submits that the text itself is not enabling and that one of ordinary skill in the art would have to conduct significant experimentation in order to perform such an analysis, thus rendering the claimed invention non-obvious.

Because the Cantor reference, by the Examiner's own admission, does not discuss providing (or comparing) the hybridization patterns from two different arrays to determine the presence of a mutation in a target polynucleotide, the Cantor reference does not cure the defect in the Southern reference.

Furthermore, the array fabrication technique disclosed in Southern is not amenable to making an n-mer array suitable for the presently claimed invention. The Southern arrays are fabricated by separating features with rubber tubing. The tubes are glued to a glass plate at 8-mm intervals (see page1009, column 1, second paragraph). The glass plate itself was 220 x 220 mm in size and allowed for a total of 256 different oligonucleotides, only enough for a 4-mer array. As the n-mers become larger, the area required to manufacture arrays with a suitable number of features using the Southern techniques becomes prohibitory.

Moreover, even if Southern does teach a method for analyzing and comparing sequences by hybridization to arrays wherein the analysis and comparison of hybridization patterns determines the presence of a mutation, applicants respectfully submit that there is no motivation to combine the teachings of Southern with the teachings of Cantor. Particularly since, as discussed above, Cantor does not teach or suggest the use of complete n-mer arrays to conduct mutation analysis, and rather, is focused on a method for replicating arrays of probes. It would not have been obvious to combine Cantor's method of replicating arrays of probes with Southern's speculative piece to produce the presently claimed invention.

Therefore, applicants respectfully submit that the Examiner has not established a prima facie case of obviousness and that the rejection to claim 1 be withdrawn.

Claims 2-11 are dependent upon Claim 1 and applicants respectfully request that the rejection of these claims be withdrawn for at least those reasons stated in the response to the rejection of Claim 1.

As to independent Claim 12, the Examiner alleges that Cantor teaches a method of determining whether two or more polynucleotides are identical (Example 13) comprising the steps of providing a probe array wherein each probe comprises a double stranded region and a single stranded n-mer overhang region such that the overhangs in each array constitute a complete set of n-mers; hybridizing a first polynucleotide to said overhangs in the array to generate a first hybridization pattern; hybridizing a second target polynucleotide to said overhangs to generate a second hybridization pattern; and determining whether the polynucleotides are identical by analyzing the hybridization patterns. The Examiner further alleges that Cantor teaches the method wherein both first and second polynucleotides are hybridized to the same array. The Examiner asserts that Cantor does not teach providing at least two identical polynucleotide probe arrays wherein the first polynucleotide is hybridized to one array and the second polynucleotide is hybridized to the second array. However, the Examiner alleges that hybridization of polynucleotides to identical probe arrays was known in the art as taught by Southern.

Applicant respectfully traverses this rejection. Applicant respectfully submits that the Examiner has mischaracterized the Cantor reference with respect to Example 13, as this section is limited to "custom arrays," which, as defined by the Cantor specification, do not contain all n-mers. Column 31, lines 2-4 state: "These do not represent all 4^{10} possible 10-mers, but instead represent just those 10-mers which were present in the original sample." (Please review applicant's response to the rejection of Claim 1, above, for further discussion.) Furthermore, as reviewed in greater detail above, applicant submits that Southern is a speculative piece which does not enable arrays of complete sets of n-mers. As stated above, the arrays of Southern comprise only oligopurines, that is, only T's and C's. While the text speculates as to the ability to create arrays comprising n-mers of all four nucleotides, the text also discusses the need for further experimentation in order to create these arrays. Southern discusses the potential problems created by foldback sequences, repeats and long homopolymer runs. (Page 1015, first column, carry-over paragraph) Furthermore, significant experimentation must

be conducted in order to accurately interpret the data generated by the arrays. The text states that the above identified problems "must be eliminated or taken into account in the interpretation of the data." (Page 1015, first column, carry-over paragraph lines 7-8.) The text goes on to suggest that the "rules are likely to be complex...(and) they will emerge from detailed analysis of experiments such as the one we used to calibrate the array of octapurines, to be reported in detail elsewhere...." (Page 1015, first column, carry-over paragraph lines 16-19.) Therefore, applicant submits that the presently claimed invention is nonobvious over the cited references as the presently claimed invention solves the problems identified in the Southern reference.

Furthermore, applicant respectfully reasserts that it would not have been obvious to combine Cantor's method of replicating arrays of probes with Southern's speculative piece to produce the presently claimed invention.

Claims 13-18 are dependent upon Claim 12 and applicant respectfully requests that the rejection of this claim be withdrawn for at least those reasons stated in the response to the rejection of Claim 12 as well as those reasons stated in the response to the rejections to Claims 2-11 as they apply to Claims 13-18, respectively.

Claim 19 is rejected as obvious over Cantor in view of Southern. Applicant has cancelled claims 19-25 without prejudice and therefore believes the rejection is mooted.

CONCLUSION

Applicant respectfully submits that the application, as amended, is in condition for allowance. Accordingly, applicant requests timely consideration of this response and consequential allowance of the claims pending in this application.

Respectfully submitted,

Date: 6/21/00

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